



Acute and repeated treatment with the 5-HT₇ receptor antagonist SB 269970 induces functional desensitization of 5-HT₇ receptors in rat hippocampus

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Abstract:

Background: SB 269970, a 5-HT₇ receptor antagonist may produce a faster antidepressant-like effect in animal models, than do antidepressant drugs, e.g., imipramine. The present work was aimed at examining the effect of single and repeated (14 days) administration of SB 269970 on the 5-HT₇ receptor in the hippocampus.

Methods: The reactivity of 5-HT₇ receptors was determined using 5-carboxamidotryptamine (5-CT), which increased the bursting frequency of spontaneous epileptiform activity in hippocampal slices. Additionally, the effects of SB 269970 administration on the affinity and density of 5-HT₇ receptors were investigated using [³H]-SB 269970 and the influence of SB 269970 and imipramine on mRNA expression levels of Gα_s and Gα₁₂ mRNA were studied using RT-qPCR.

Results: Acute and repeated treatment with SB 269970 led to attenuation of the excitatory effects of activation of 5-HT₇ receptors. Neither single nor repeated administration of SB 269970 changed the mean affinity of 5-HT₇ receptors for [³H]-SB 269970. Repeated, but not single, administration of SB 269970 decreased the maximum density of [³H]-SB 269970 binding sites. While administration of imipramine did not change the expression of mRNAs for Gα_s and Gα₁₂ proteins after both single and repeated administration of SB 269970, a reduction in Gα_s and Gα₁₂ mRNA expression levels was evident.

Conclusions: These findings indicate that even single administration of SB269970 induces functional desensitization of the 5-HT₇ receptor system, which precedes changes in the receptor density. This mechanism may be responsible for the rapid antidepressant-like effect of the 5-HT₇ antagonist in animal models.

Key words:

5-carboxamidotryptamine, adaptive changes, epileptiform activity, hippocampal slice, imipramine, SB 269970

Introduction

Serotonin (5-hydroxytryptamine, 5-HT), which acts as a neurotransmitter and/or a neuromodulator, is involved in a wide spectrum of physiological processes including sleep, cognition, sensory perception, motor

activity, temperature regulation, appetite, hormone secretion, nociception, and sexual behavior (reviewed in: [30]). Dysfunctions of the serotonergic system are thought to be involved in the pathomechanism of depressive disorders. Besides other structures, the hippocampus plays an important role as a target for anti-

depressant and anxiolytic drugs [72] (reviewed in: [40, 47]). It has been suggested that a common result of different types of antidepressant therapies is an enhancement of 5-HT neurotransmission within the hippocampus (reviewed in: [9, 19, 27]).

The cellular effects of 5-HT are mediated by up to 14 distinct membrane receptor subtypes that may be expressed in various amounts in single neurons (reviewed in: [28, 29, 55]). Such a diversity permits the occurrence of different effects of 5-HT, including both, inhibitory and excitatory influence on neuronal networks. These mechanisms allow 5-HT to remodel neuronal excitability in a variety of cell types and neuronal circuits in a functionally appropriate manner. In the hippocampus, the most prominent modulatory effect of 5-HT is a 5-HT_{1A} receptor-mediated reduction of the excitability of pyramidal cells [1]. Another 5-HT receptor subtype which effectively modulates neuronal activity is the 5-HT₄ receptor whose activation increases excitability of hippocampal pyramidal cells [12, 15]. Adaptive modifications of serotonergic mechanisms modulating the functions of forebrain structures provide an effective mechanism of antidepressant therapies (reviewed in: [8, 36, 39]). In rats, repeated administration of tricyclic antidepressants (TCAs) enhances the inhibitory effect of 5-HT_{1A} receptor activation on the excitability of hippocampal pyramidal neurons [6, 13, 17, 35]. Adaptive changes induced by treatment with the TCA imipramine in rat hippocampus involve attenuation of the excitatory effect of 5-HT₄ receptor activation [7, 74]. Repeated administration of selective serotonin reuptake inhibitors (SSRIs) reduces the effectiveness of hippocampal 5-HT₄ receptor activation as well; however, at variance with the effects of TCA, the sensitivity of hippocampal 5-HT_{1A} receptors remains unchanged after treatment with SSRIs [7, 13, 14, 62] (reviewed in: [27]).

The 5-HT₇ receptor is the latest 5-HT receptor subtype to be identified [4, 54]. In the brain, the 5-HT₇ receptor is predominantly expressed in the thalamus, hippocampus, hypothalamus [48] and raphe nuclei [37]. This receptor has been implicated in mood regulation, circadian rhythmicity and sleep, the disturbances of which are evident in the course of affective disorders (reviewed in: [26, 61]). It has been well established that neuronal 5-HT₇ receptors activate adenylate cyclase through G α_s protein [34, 56]. Interestingly, it has been shown that activation of the 5-HT_{7A} receptor stimulates AC1 and AC8 Ca²⁺/calmodulin-dependent isoforms of adenylate cyclase which are in-

sensitive to G α_s *in vivo* [3]. Moreover, it has been found that 5-HT₇ receptors may also activate G α_{12} protein [32]. On a cellular level, activation of the 5-HT₇ receptor decreases potassium conductances and increases the hyperpolarization-activated current I_h, and thus enhances the excitability of hippocampal pyramidal cells [2, 5, 65]. All these effects contribute to the 5-HT₇ receptor-mediated facilitation of hippocampal population spikes *in vivo* [38], as well as to the enhancement of epileptiform activity in disinhibited hippocampal slices *in vitro* [20, 49, 64].

It has been suggested that the modification of 5-HT₇ receptor activity resulting from chronic treatment with antidepressants may represent a mechanism underlying the therapeutic effect of these drugs [57] (reviewed in: [24, 26]). It is noteworthy that several psychotropic drugs exhibit high affinity for 5-HT₇ receptors [50, 52, 56, 59] (reviewed in: [33]). It has also been shown that certain antidepressants may exert some effects by acting directly on the 5-HT₇ receptor [46].

Recent studies have demonstrated a synergistic interaction between serotonin receptors antagonists and several antidepressant drugs [53, 67], including the specific 5-HT₇ receptor antagonist SB 269970 [11, 70]. In animal models, inactivation or blockade of the 5-HT₇ receptor has been shown to induce antidepressant-like behavior [21, 25, 69]. Chronic treatment with antidepressants has also been shown to modify the reactivity of 5-HT₇ receptors. The downregulation of the 5-HT₇ receptor has been found to take place in rat suprachiasmatic nucleus of the hypothalamus after chronic treatment with TCAs including imipramine, and the SSRI – fluoxetine [46, 58]. Our earlier study indicated attenuation of the effects of activation of rat hippocampal 5-HT₇ receptors after treatment with the TCA imipramine and the SSRI citalopram [64]. These findings support the hypothesis that the 5-HT₇ receptor may be a target for the action of antidepressant drug. Moreover, blockade of this receptor opens up good possibilities for the treatment of depression [43] (reviewed in: [24, 40, 44]). It is widely known that the response to treatment with conventional antidepressants may be delayed for several weeks.

Previously, our electrophysiological study showed that repeated (14 times), but not single, imipramine administration diminished the reactivity of the 5-HT₇ receptor [64]. We also demonstrated that chronic treatment with imipramine modified neither the affinity of 5-HT₇ receptors to [³H]-SB 269970 nor the density of those receptors [63].

Recent research has shown that acute administration of SB 269970, a 5-HT₇ receptor antagonist, shortens immobility time in the forced swim test [68]. Moreover, blockade of the 5-HT₇ receptor may produce a faster antidepressant effect than do contemporary antidepressant drugs [42].

Since only the effect of acute administration of SB 269970 has been investigated so far (mainly in behavioral studies), the present work was aimed at examining the effect of acute and prolonged treatment with SB 269970 on the reactivity of the 5-HT₇ receptor in the hippocampus using biochemical and electrophysiological methods. We studied the influence of the blockade of 5-HT₇ receptors on their affinity and density. The 5-HT₇ receptor may be coupled to two different G proteins: G α_s and G α_{12} (reviewed in: [71]). For this reason we also examined the effect of SB 269970 on G α_s and G α_{12} mRNAs expression and compared the impact of 5-HT₇ receptor blockade with the effect of a the classic tricyclic antidepressant (TCA) imipramine.

Materials and Methods

Drugs

5-Carboxamidotryptamine maleate (5-CT), (2R)-1-[(3-hydroxyphenyl)sulfonyl]-2-[2-(4-methyl-1-piperidinyl)ethyl]pyrrolidine hydrochloride (SB 269970) and N-{2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl}-N-2-pyridinylcyclohexanecarboxamide (WAY 100635) were obtained from Sigma-Aldrich. [³H]-SB 269970 (62.7 Ci/mol) was purchased from Moravex Biochemicals, Brea, CA.

Treatment of animals

The experimental procedures were approved by the Animal Care and Use Committee at the Institute of Pharmacology, PAS and were carried out in accordance with the "Principles of laboratory animal care" (NIH publication no. 85-23, revised 1985) and the national law. Male Wistar rats, weighing approx. 100 g at the beginning of the experiment, were housed in groups on a controlled light/dark cycle (the light on: 7:00–19:00) and had free access to standard food and tap water. The rats received SB 269970 (1.2 mg/kg,

ip, dissolved in 0.9% NaCl, volume: 1 ml/kg) either repeatedly for 14 days, or as a single injection. In the latter group, single administration of SB 269970 was preceded by 13 daily injections of 0.9% NaCl. The animals of the control group received 0.9% NaCl once daily for 14 days.

Two separate experimental groups of the animals received imipramine (10 mg/kg, *ip*, dissolved in 0.9% NaCl, volume: 1 ml/kg) once daily for 14 consecutive days, or as a single injection. In the latter group single administration of imipramine was preceded by 13 daily injections of 0.9% NaCl. The animals of the control group for imipramine experiments received 0.9% NaCl once daily for 14 days.

The rats were killed by decapitation two days after the last drug administration; then their brains were removed and used for further analyses.

Slice preparation, electrophysiological recording and data analysis

The brains were immersed in an ice-cold artificial cerebrospinal fluid (ACSF) composed of (in mM): NaCl (124), KCl (5), CaCl₂ (2.5), MgSO₄ (1.3), KH₂PO₄ (1.25), NaHCO₃ (24) and D-glucose (10). ACSF was bubbled with a mixture of 95% O₂/5% CO₂. After dissection, the hippocampus was cut into transverse slices (400 μ m thick) using a vibrating microtome (Leica, USA).

The slices were left to recover in the holding chamber at 32 \pm 0.5°C, for 1–6 h. A single slice was then transferred to the recording chamber of a submerged type and was superfused (1.5 ml/min) with a warmed (32 \pm 0.5°C), modified ACSF, in which [NaCl] was raised to 132 mM and [KCl] was lowered to 2 mM, devoid of Mg²⁺ ions. Glass micropipettes filled with 2 M NaCl (1–4 M Ω) were used to record activity from the pyramidal layer of the CA3 area. Spontaneous epileptiform bursts were amplified (Axoprobe 2, Axon Instruments, USA), band-pass filtered (1 Hz – 10 kHz), A/D converted, stored on a PC (1401 interface with Signal 2 software, CED, UK) and analyzed off-line [66]. Drug effects were assessed in terms of changes in bursting frequency (\pm SEM) and by comparing the average frequency over 6–10 min after the beginning of 5-carboxamidotryptamine maleate (5-CT) application to the baseline values (see Fig. 1). The data from treated and control rats were also compared using a paired *t*-test.

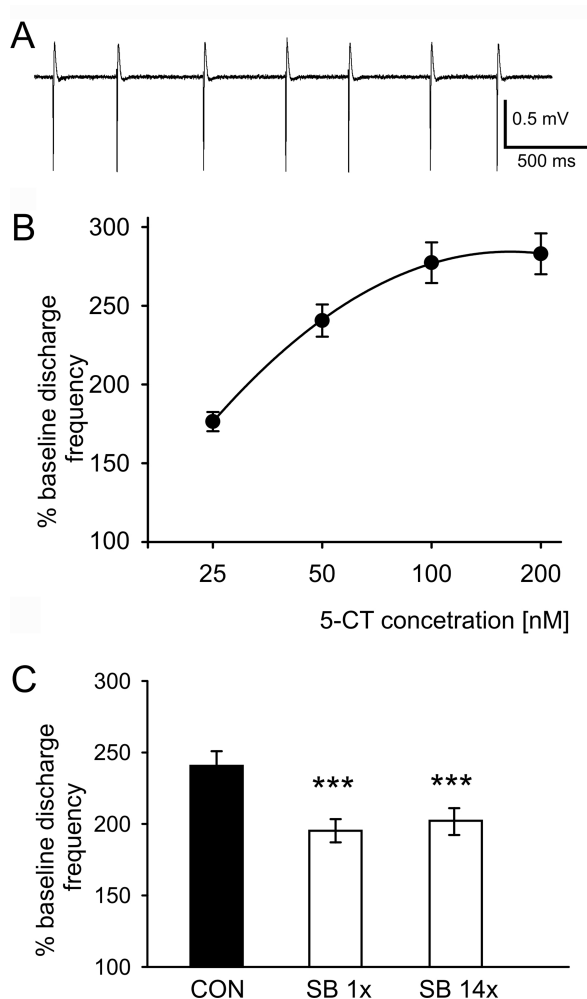


Fig. 1. The influence of administration of the 5-HT₇ receptor antagonist SB 269970 on the 5-HT₇ receptor-mediated excitatory effect of 5-CT in *ex vivo* hippocampal slices. **(A)** Spontaneous bursting activity recorded in the CA3 area in a representative experiment. **(B)** A dose-response curve for the effect of 5-CT on the bursting activity in control preparations (the mean \pm SEM). **(C)** Single (labeled: SB 1x) and repetitive (labeled: SB 14x) administration of SB 269970 resulted in attenuation of the excitatory effect of 50 nM 5-CT compared to slices obtained from untreated rats (CON). *** $p < 0.001$, t -test

Membrane preparation and saturation analysis

Rat hippocampi were immediately frozen on dry ice and stored at -80°C . The membranes were prepared according to the method described previously [10] by homogenizing (Ultra Turrax) the tissue in 20 volumes (based on wet weight) of 50 mM Tris-HCl (pH = 7.4 at 37°C). Following centrifugation ($50,000 \times g$, 12 min, 4°C) the pellets were resuspended in the same medium and incubated at 37°C for 15 min. After a fur-

ther three centrifugation and resuspension steps, the pellets were stored at -80°C for further analysis. Saturation binding assays were performed using [^3H]-SB 269970 according to the method described by Thomas et al. [60]. On the day of the experiment, the membranes (approx. 15 mg tissue/tube) were defrosted, suspended in a Tris-HCl buffer (50 mM, pH = 7.4 at 37°C) containing CaCl_2 (4 mM), pargyline (0.1 mM) and ascorbic acid (1 mM) and were incubated with [^3H]-SB 269970 (eight concentrations within a range of: 0.2–11 nM) for 60 min at 37°C . The non-specific binding was determined using 10 μM 5-HT. The incubation was terminated by passing through Whatman GF/B filters, followed by immediate washing with an ice-cold Tris-HCl buffer. The bound radioactivity remaining on the filters was assayed by liquid scintillation spectroscopy (Beckman L SM 6500). All the assays were performed in triplicate in three separate experiments. The binding data were analyzed using non-linear regression (GraphPad Software Inc., San Diego, USA) generating K_d and B_{max} values.

RNA isolation and RT-qPCR

A frozen hippocampal tissue was placed in the Lysis Buffer (4.5 M guanidine-HCl, 100 mM sodium phosphate, pH 6.6; Roche, Germany) at a volume of 0.4 ml/20 mg of the tissue, and was homogenized by high-speed shaking (30/s) in plastic tubes with stainless steel beads in the TissueLyserII apparatus (Qia-gene, USA). Total RNA was purified using a High Pure RNA Tissue Kit (Roche, Germany) according to the manufacturer's protocol. The quantity of RNA was determined spectrophotometrically at 260 nm and 260/280 nm (ND/1000 UV/Vis; Thermo Fisher Nano-Drop, USA) and its quality was confirmed by electrophoresis on agarose gel.

A two-step reaction: a reverse transcription (RT) and a quantitative polymerase chain reaction (qPCR) was run in the Chromo4 RealTime PCR Instrument (MJ Research, USA). Five hundred nanograms of total RNA and 2 U of RNase inhibitor (Fermentas, Lithuania) were incubated for 5 min at 65°C and chilled on ice. An RT reaction was performed at a final volume of 20 μl of the reaction mixture containing 1 \times AMV reverse transcriptase buffer (Finnzymes, Finland), 1 mM deoxynucleotide-3-phosphate mixture (dNTP, Fermentas), 10 μM universal primer oligo (dT), 2 U of the ribonuclease inhibitor (Ribolock, Fermentas, Lithuania), 10 U of AMV reverse transcrip-

tase. The RT reaction was carried out at 42°C for 90 min and was followed by a denaturation step at 70°C for 10 min. The products of RT reaction were amplified using TaqMan probes and primers for the $G\alpha_s$, $G\alpha_{12}$ and hypoxanthine-guanine phosphoribosyltransferase (HPRT) (Assays ID: Rn00569454_m1 and Rn00578965_m1 and Rn01527840_m1, respectively) (Applied Biosystems) and a FastStart Universal Probe Master (Rox) kit (Roche, Germany). Amplification was carried out at a total volume of 10 μ l of the reaction mixture containing 1 \times FastStart Universal Probe Master (Rox) mix (Roche, Germany), 50 ng of cDNA as a PCR template, 900 nM TaqMan forward and reverse primers and 250 nM hydrolysis probe labeled with the fluorescent reporter dye FAM at the 5'-end, and a quenching dye at the 3'-end. The qPCR was run with the following profile: 1 cycle at 50°C for 2 min, 1 cycle at 95°C for 10 min and 40 cycles each at 95°C for 15 s and 60°C for 1 min. The threshold value (C_t) for each sample was set in the exponential phase of PCR, and the $\Delta\Delta C_t$ method was used for data analysis. HPRT was used as reference gene, and its expression was observed at a constant level in each experimental group of the animals.

Statistical analysis

All the values are given as the mean \pm standard error of the mean (SEM). A statistical analysis of the data was performed using Statistica 8.0 software (StatSoft, Tulsa, USA) and a one-way analysis of variance (ANOVA), followed by Fisher's Least Significant Difference (LSD) test, $p < 0.05$ was considered statistically significant. The data from treated and control rats were also compared using a paired t -test.

Results

Effect of SB 269970 administration on bursting activity

Epileptiform bursting of a regular frequency occurred within 15–20 min after the placement of slices in a nominally Mg^{2+} -free, modified ACSF. Individual bursting events consisted of an initial, population spike-like waveform (1–2 mV in amplitude), which was followed by a slower, positive-going wave. As re-

Tab. 1. The influence of single (SB 1 \times) and repeated (SB 14 \times) administration of SB 269970 on the mean (\pm SEM) basal discharge frequency in *ex vivo* hippocampal slices

	Basal discharge frequency [Hz]	<i>n</i>
CON	0.116 \pm 0.0189	46
SB 1 \times	0.113 \pm 0.0146	30
SB 14 \times	0.0556 \pm 0.0072*	32

* $p < 0.013$, SB 14 \times vs. control

ported previously, the application of 5-CT to the ACSF in the presence of WAY 100635 resulted in a dose-dependent, 5-HT₇ receptor-mediated increase in the bursting frequency which reached its maximum between 6–10 min after the start of 5-CT application [49, 64]. Single administration of SB 269970 did not change the mean baseline bursting frequency, which did not differ from that recorded in slices obtained from the control groups of animals receiving the vehicle (Tab. 1). However, repeated administration of SB 269970 resulted in a ca. twofold decrease in the baseline bursting frequency (Tab. 1).

To investigate the effects of SB 269970 administration on the 5-HT₇ receptor-mediated enhancement of the bursting activity, 50 nM 5-CT was applied to the ACSF. As shown in Figure 1C, the magnitude of the 5-CT-induced effect was attenuated in hippocampal slices prepared from the animals receiving single as well as repeated doses of the 5-HT₇ receptor antagonist.

Effect of SB 269970 administration on the affinity and density of 5-HT₇ receptors

Neither single nor repeated administration of SB 269970 changed the mean affinity of 5-HT₇ receptors for [³H]-SB 269970. After single administration, the maximum density (B_{max}) of the receptors also re-

Tab. 2. The influence of single (SB 1 \times) and repeated (SB 14 \times) administration of SB 269970 on the mean (\pm SEM) affinity (pK_d) and the maximum density (B_{max} ; \pm SEM) of 5-HT₇ receptors in rat hippocampus

	CON	SB 1 \times	SB 14 \times
pK_d	0.8.99 \pm 0.06	8.78 \pm 0.05	9.04 \pm 0.06
B_{max} [pM/mg]	0.14 \pm 0.003	0.13 \pm 0.005	0.09 \pm 0.004 [#]

[#] $p < 0.001$, SB 14 \times vs. control

mained unchanged (Tab. 2). On the contrary, after 14 injections of the 5-HT₇ receptor antagonist, the value of B_{max} significantly decreased (Tab. 2).

Effect of imipramine and SB 269970 on G-protein mRNA expression

Neither single nor repeated administration of imipramine induced changes in the expression of mRNAs for the G proteins studied (Figs. 2A₂, B₂). In contrast, SB 269970 injections caused a statistically significant reduction in Gα_s and Gα₁₂ mRNA expression levels. Those effects were evident after both single and repeated administration of the 5-HT₇ receptor antagonist (Figs. 2A₁, B₁).

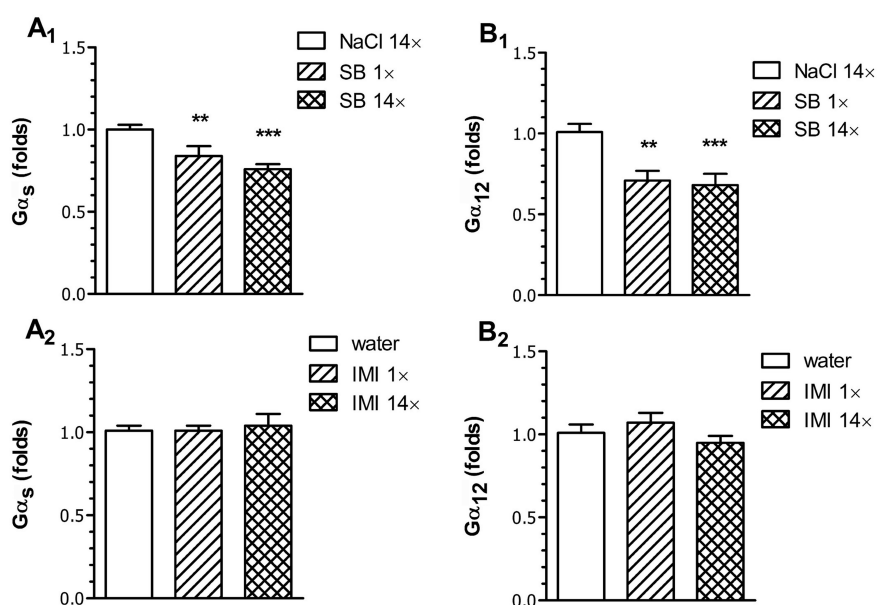
Discussion

The major finding of this study is that treatment with a specific 5-HT₇ receptor antagonist decreases the reactivity of the 5-HT₇ receptor, which can be seen after both single and repeated administration. For the last several years, a number of studies have attempted to evaluate the role of the 5-HT₇ receptor in depressive disorders, as well as in the action of antidepressant drugs. It has recently been shown that the block-

ade of the 5-HT₇ receptor synergistically potentiates the effect of clinically used antidepressants in an animal model of depression [70]. Moreover, it has been demonstrated that the antagonist of the 5-HT₇ receptor *per se* can exert antidepressive action [42]. The above data suggest that the blockade of the 5-HT₇ receptor may play a role in the antidepressive action, and that some antidepressants can exert their therapeutic effect *via* blockade of the 5-HT₇ receptor or *via* a decrease in 5-HT₇ receptor reactivity. The results of our earlier study showing that chronic treatment with the antidepressants of different classes may decrease the reactivity of the 5-HT₇ receptor seem to support this assumption [64].

The present study provides evidence for attenuation of the excitatory effect of activation of the 5-HT₇ receptor after treatment with SB 269970. In contrast to several antidepressants tested so far, this effect occurred not only after repeated treatment, but already after single injection of the antagonist. The swift action of SB 269970 on the reactivity of the 5-HT₇ receptor is in line with the results obtained by other authors. Mnie-Filali and coworkers [42] found a reduced 5-HT_{1A} and/or 5-HT₇ receptor responsiveness in the dorsal raphe nucleus (DRN) after one week of 5-HT₇ receptor blockade. To achieve a similar effect for standard antidepressants, at least 21-day treatment is necessary. Thus, the pharmacological blockade of 5-HT₇ receptors produces a considerably faster

Fig. 2. The influence of treatment with the 5-HT₇ receptor antagonist SB 269970 and imipramine on the expression of Gα_s and Gα₁₂ mRNA in the hippocampus. **(A)** The Gα_s mRNA expression level after single (1×) or repeated (14×) administration of SB 269970 (labeled SB; **A₁**) and imipramine (labeled: IMI; **A₂**). **(B)** The Gα₁₂ mRNA expression level after single (1×) or repeated (14×) administration of SB 269970 (labeled SB; **B₁**) and imipramine (labeled: IMI; **B₂**). **p* < 0.001, ****p* < 0.0001 vs. vehicle (either NaCl or water); ANOVA, *n* = 9–10 per group



antidepressant-like response than do most antidepressant drugs [42]. Such a phenomenon may occur as a result of either an increased 5-HT₇ receptor activation or activation of other 5-HT receptors by an elevated extracellular 5-HT level due to the enhanced activity of serotonergic neurons. It has been shown that the blockade of 5-HT₇ receptors increases 5-HT raphe–hippocampus transmission [11]. Enhancement of 5-HT transmission in such projection areas as the dorsal hippocampus after 1 week of SB 269970 administration has also been reported by other researchers [42]. On the other hand, some authors have found that the 5-HT₇ receptor antagonist SB 269970 significantly inhibits 5-HT efflux [51]; however, other investigators have shown that inhibition of 5-HT release is likely to be mediated by 5-HT₇ receptor agonists [22]. The mechanism of control of DRN neuronal activity by 5-HT₇ receptors seems to be indirect, since the regulation of DRN activity *via* the 5-HT₇ receptor is tetrodotoxin-sensitive [23]. This may suggest that 5-HT₇ receptors are not directly localized on 5-HT neurons, but rather on GABAergic and/or glutamatergic ones [23, 45]. It is speculated that the local glutamatergic, GABAergic and serotonergic circuitry in the raphe nuclei forms an excitatory-inhibitory connection by which incoming excitatory signals are converted into an inhibitory output projecting to various brain areas such as, e.g., cerebral cortex, striatum, hippocampus or hypothalamus [23, 45].

Alternatively, the decreased reactivity of hippocampal 5-HT₇ receptors may result from a direct interaction between SB 269970 molecules and the receptor. The molecular mechanism underlying the observed attenuation of the excitatory effect of activation of the 5-HT₇ receptor after treatment with SB 269970 may be related either to the decreased receptor density or modifications in the capacity of the receptor to activate G protein, changes in G protein expression or phosphorylation, or modifications at the level of effectors (reviewed in: [18, 27]). The downregulation of the 5-HT₇ receptor, related to chronic treatment with a variety of antidepressants including imipramine and fluoxetine, was previously found to occur in the suprachiasmatic nucleus of rat hypothalamus [46, 58], where such treatment reduced 5-HT₇ receptor density by approx. 30%, without changing the receptor affinity, though.

As mentioned above, the SB 269970-induced decrease in the reactivity of rat CA1 hippocampal neu-

rons to 5-HT₇ receptor activation may also be related to modifications in the transduction pathway, including changes in the receptor density and/or in the coupling of the receptor to G protein. In fact, our data demonstrate that both single and repeated administration of SB 269970 reduces the level of mRNA of 5-HT₇ receptor coupled G proteins ($G\alpha_s$ and $G\alpha_{12}$). It should be stressed that these changes are specific to the application of the 5-HT₇ antagonist, since imipramine administered once or repeatedly does not alter the level of the mRNAs. We previously showed that repeated administration of imipramine (lasting 14 days) decreased the responsiveness of 5-HT₇ receptors in the CA3 area and that imipramine did not modify the mean basal bursting frequency [64]. However, the present data show that repeated administration of SB 269970 decreases the mean basal bursting frequency, which is lower when compared to the activity recorded in slices obtained from control animals. The most likely explanation of such an effect is reduction of excitatory synaptic transmission. The above results suggest that changes induced by chronic or prolonged administration of SB 269970 depend, at least in part, on mechanisms different from those underlying changes caused by imipramine. It has been demonstrated that activation of 5-HT₇ receptors increases neurite outgrowth in hippocampal neurons [32]. Both $G\alpha_s$ and $G\alpha_{12}$ proteins can regulate cellular morphology by activating different signaling cascades. $G\alpha_s$ protein-mediated morphogenic effects are produced by either modulation of cAMP concentration [16] or direct binding of $G\alpha_s$ protein to the cytoskeleton [73]. The downstream effectors of $G\alpha_{12}$ protein, which mediate changes in the actin cytoskeleton, are members of the Rho family of small GTPases, including RhoA, Rac1 and Cdc42 (reviewed in: [31]). The major functional effects of this pathway, including actin reorganization and the formation of neurite-like protrusions, are mediated by the activation of Cdc42 [32]. It is proposed that activation of the 5-HT₇ receptor by $G\alpha_s$ and $G\alpha_{12}$ proteins may stimulate glutamatergic synaptic transmission by a positive influence on a number of functional synapses. Hence, the prolonged blockade of the 5-HT₇ receptor, resulting in a decreased expression of $G\alpha_s$ and $G\alpha_{12}$ proteins might account for the observed reduction of glutamatergic transmission.

All in all, the current study shows that the blockade of the 5-HT₇ receptor by SB 269970 leads to functional desensitization of the 5-HT₇ receptor system at

a level of its reactivity and effector proteins, although changes in the receptor density occur after chronic treatment with the antagonist only. The phenomenon of 5-HT₇ receptor system downregulation may be an important factor in the mechanism of the antidepressant effect of the 5-HT₇ antagonist, as well as in the modulation of reactivity of other neurotransmitter systems.

Conflict of interest:

The authors declare no conflict of interest.

Acknowledgments:

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